Australasian Plant Dis. Notes (2016) 11: 35
DOI 10.1007/s13314-016-0223-4

Confirmation of *Paracercospora egenula* causing leaf spot of eggplant in Hawaii

Niloofar Vaghefi[1] · **Sarah J. Pethybridge**[1] · **Roger G. Shivas**[2] · **Scot C. Nelson**[3]

Received: 3 August 2016 / Accepted: 2 November 2016 / Published online: 10 November 2016

Abstract Morphological and molecular studies confirmed *Paracercospora egenula* as the primary cercosporoid fungus causing leaf spot of eggplant (*Solanum melongena*) in Hawaii. This is the first confirmed record of *P. egenula* in Hawaii, although it was previously reported as *C. solani-melongenae*, based on an interception at Oahu, Hawaii, in 1946. Another cercosporoid species, *Cercospora melongenae*, reported to cause leaf spot on eggplant in Hawaii, was not detected.

Keywords Cercosporoid · *Cercospora melongenae* · *Paracercospora egenula* · Hawaii

Leaf spot of eggplant (*Solanum melongena*) is prevalent in vegetable gardens in Honolulu, Hawaii, with disease incidence reaching almost 100 % in the Manoa Valley, Oahu. The disease causes necrotic, angular or circular to oblong lesions of up to 7 mm in diam., occasionally with shot-holes in the centers (Fig. 1). Lesions may coalesce under favourable conditions and cause severe defoliation. Multiple cercosporoid species have been reported to cause leaf spot on eggplant worldwide. In his monograph of the genus *Cercospora*, Chupp (1954) listed *C. deightonii*, *C. melongenae* and *C. solani-melongenae* from eggplant. The only cercosporoid species recorded in the Hawaiian and USDA databases on eggplant in Hawaii is *Cercospora melongenae* (Anonymous 1960; Raabe et al. 1981). Deighton (1979) listed *C. solani-melongenae* as a heterotypic synonym of *Paracercospora egenula*. One of the herbarium specimens (IMI 90164 = BPI 441400) that Deighton (1979) studied was from eggplant intercepted at Oahu, Hawaii, and originally identified as *C. solani-melongenae* by Flora G. Pollack in 1946. Subsequently, Crous and Braun (2003) listed Hawaii in the geographical distribution of *P. egenula*, probably because Deighton (1979) had listed Hawaii as the origin of the intercepted specimen (IMI 90164). This specimen, however, was not subsequently listed in the Hawaiian and USDA databases as present in Hawaii, most likely because it represented a quarantine interception from another country. Our objective was to confirm the identity of the cercosporoid fungus associated with leaf spot of eggplant in Hawaii through morphological and molecular characterisation.

In November 2015, 13 isolates of cercosporoid fungi were collected from leaves of several varieties of eggplant at five community gardens at four locations (Manoa Valley, Ala Wai, Diamond Head and Makiki) in Honolulu, Hawaii. Conidia from individual lesions were collected by pipetting 50 µl of T-water (0.06 % *v*/v Tween-20 + 0.02 % *w*/*v* filter-sterilized ampicillin) onto the surface of sporulating lesions and transferring conidial suspensions onto water agar amended with ampicillin (0.02 % *w*/*v*). After 24 h incubation at room temperature, germinated conidia were transferred to Petri plates containing clarified V8-media (10 % *v*/v clarified V8 juice, 0.5 % $CaCO_3$, 1.5 % *w*/*v* agar). Four monosporic isolates representing each of the four locations (HI-022, HI-027, HI-028, and HI-033; Table 1) were grown on potato dextrose agar (PDA), malt extract agar (MEA) and oatmeal agar (OA) for cultural characterisation.

✉ Scot C. Nelson
snelson@hawaii.edu

[1] School of Integrative Plant Science, Plant Pathology & Plant-Microbe Biology Section, Cornell University, Geneva, NY 14456, USA

[2] Plant Pathology Herbarium, Department of Agriculture and Fisheries, Dutton Park, QLD 4102, Australia

[3] College of Tropical Agriculture and Human Resources, Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu, HI 96822, USA

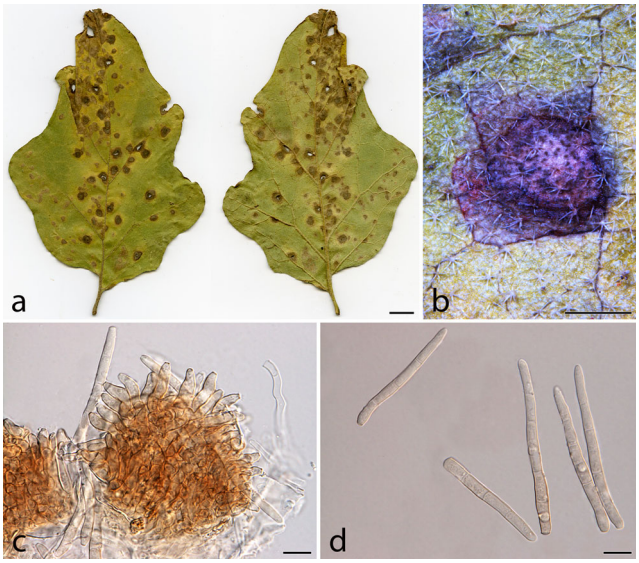

Fig. 1 **a** Cercospora leaf spot symptoms on upper (*left*) and lower (*right*) surfaces of an eggplant leaf. **b** lesion with conidiomata on upper leaf surface. **c** conidiophores on conidioma**. d** conidia. Scale bars =1 cm (**a**), 1 mm (**b**), 10 μm (**c**), 10 μm (**d**)

Genomic DNA was extracted from isolates HI-022, HI-027, HI-028, and HI-033 using a QIAGEN DNeasy Plant Mini Kit according to the manufacturer's instructions, from lyophilized mycelia derived from cultures grown in clarified V8 broth (10 % *v*/v clarified V8 juice, 0.5 % *w*/*v* CaCO3) on a shaker at 100 rpm at room temperature for 7 days. Standard primers were used to amplify the sequences of the ITS (White et al. 1990), LSU (Rehner and Samuels 1994), partial actin (ACT; Carbone and Kohn 1999), and translation elongation factor (EF-1α; Carbone and Kohn 1999), and all the sequences were deposited in the NCBI GenBank nucleotide database (Table 1). Maximum likelihood (ML) analysis was conducted on the concatenated alignment of the four loci (LSU: 1265; ITS: 488; EF-1α: 281; ACT: 189; total 2223), in RAxML v. 7.2.6 (Stamatakis 2006) using the GTRGAMMA model applied to the individual partitions with 1000 bootstraps. The resulting ML tree was viewed in TreeView v. 1.6.6. (Page 1996).

Mean colony diam. of the isolates ranged from 22 to 26 mm on PDA and OA; and from 21 to 27 mm on MEA, after incubation in the dark at room temperature (23–25 °C) for 6 weeks. Leaf spots amphigenous, scattered, circular to angular, 1–7 mm diam, with pale to dark brown centres, surrounded by diffuse chlorotic haloes. Conidiomata sporodochia-like, epiphyllous, up to 80 μm diam, pale to olivaceous brown. Conidiophores in dense fascicles arising from the upper cells of the conidiomata, reduced to conidiogenous cells or 1-septate, subcylindric, narrowed towards the tip, 10–25 × 3–5 μm, pale brown, smooth, conidiogenous loci apical and flattened with a circular darkened-refractive rim. Conidia cylindrical to obclavate, 25–75 × 3.5–5.5 μm, straight or curved to flexuous, apex obtuse, base obconically truncate, 2–6 septate, smooth, subhyaline to pale brown (Fig. 1), which was similar to the description of *Paracercospora egenula* (Deighton 1979; Crous et al. 2013). A representative culture has been lodged with the International Collection of Microorganisms from Plants, Auckland, New Zealand (ICMP 21323) and a herbarium specimen with the Queensland Plant Pathology Herbarium, Brisbane, Australia (BRIP 64766).

Paracercospora is characterized by circular conidiogenous loci with slightly thickened, darkened-refractive rims and pale conidia (Braun et al. 2013). It was recovered as a monophyletic genus sister to *Pseudocercospora* (Crous et al. 2013). The LSU and ITS sequences of all Hawaiian isolates were identical to that of *P. egenula* MUCC 883 and CBS 485.81 but 2 and 1 bp different from the LSU and ITS of CBS 132030, respectively. The ACT sequences were identical to

Table 1 *Paracercospora egenula* isolates obtained from eggplant in Hawaii and reference isolates included in the morphological and/or phylogenetic analyses

Strain no.[a]	Country	Collector	Genbank accessions				Reference
			LSU	ITS	EF-1α	ACT	
CBS 485.81	India	N. Ponnapa	JQ324940	GU269699	GU384415	GU320400	Crous et al. (2013)
CPC 12537 = CBS 132030	South Korea	H.D. Shin	GU253738	GU269698	GU384414	GU320402	Crous et al. (2013)
HI-022 = ICMP 21323	HI, USA	N. Vaghefi	KX212318	KX212314	KX212322	KX212310	This Study
HI-027	HI, USA	N. Vaghefi	KX212319	KX212315	KX212323	KX212311	This Study
HI-028	HI, USA	N. Vaghefi	KX212320	KX212316	KX212324	KX212312	This Study
HI-033	HI, USA	N. Vaghefi	KX212321	KX212317	KX212325	KX212313	This Study
MUCC 883	Japan	T. Mikami	GU253739	GU269700	GU384416	GU320404	Crous et al. (2013)

[a] CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC Culture collection of Pedro Crous, housed at CBS; ICMP International Collection of Microorganisms from Plants, New Zealand; MUCC Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan

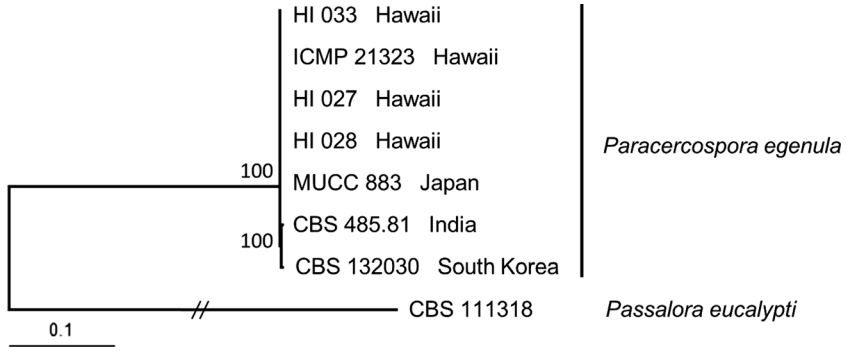

Fig. 2 Reconstructed phylogeny of *Paracercospora egenula* isolates from *Solanum melongena*, based on Maximum Likelihood (ML) analysis of the combined LSU, ITS, EF-1α and ACT sequences. The bootstrap support values of the ML analysis are given above the branch. The bar indicates the number of substitutions per site. The tree is rooted with *Passalora eucalypti* (CBS 111318 = CPC 1457)

those of MUCC 883, CBS 485.81 and CBS 132030. The EF-1α sequences were identical to MUCC 883 and 2 bp different from CBS 485.81 and CBS 132030. Maximum likelihood analysis showed that Hawaiian isolates formed a highly supported clade with the three *P. egenula* reference cultures sequenced by Crous et al. (2013) (Fig. 2). *Paracercospora egenula* was confirmed as the primary cercosporoid fungus causing leaf spot of eggplant in Hawaii while *C. melongenae*, previously reported on eggplant in Hawaii, was not detected.

Acknowledgments This research was supported by the United States Department of Agriculture, National Institute of Food and Agriculture Hatch project NYG-625424, managed by the New York Agricultural Experiment Station, Cornell University, Geneva, NY. Thanks to Mr. David Strickland for excellent technical support.

References

Anonymous (1960) Index of Plant Diseases in the United States. US Dep Agric Handb 165:1–531

Braun U, Nakashima C, Crous PW (2013) Cercosporoid fungi (*Mycosphaerellaceae*) 1. Species on other fungi, *Pteridophyta* and *Gymnospermae*. IMA Fungus 4:265–345

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Chupp C (1954) A monograph of the fungus genus *Cercospora*. Ithaca, New York, USA

Crous PW, Braun U (2003) *Mycosphaerella* and its anamorphs. 1. Names published in *Cercospora* and *Passalora*. CBS Biodiversity Series, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Crous PW, Braun U, Hunter GC, Wingfield MJ, Verkley GJM, Shin HD, Nakashima C, Groenewald JZ (2013) Phylogenetic lineages in *Pseudocercospora*. Stud Mycol 75:37–114

Deighton FC (1979) Studies on *Cercospora* and allied genera. VII. New species and redispositions. Mycol Pap 144:1–56

Page RDM (1996) Treeview: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Raabe RD, Conners IL, and Martinez AP (1981) Checklist of plant diseases in Hawaii. College of Tropical Agriculture and Human Resources, University of Hawaii. Information Text Series No. 22. Hawaii Inst. Trop. Agric. Human Resources, 313 pp

Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res 98:625–634

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols. A guide to methods and applications. Academic, San Diego, pp. 315–322